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Analytical Review on UV Spectroscopy

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ABSTRACT

There are growing variety of multicomponent formulations, biotherapeutic products, and complex matrix samples in question, quick and simple analytical strategies square measure are required. For this purpose, a variety of Ultraviolet (UV) spectrophotometric strategies are used. Different types of uv spectrometric method developed on the basis of principle of additivity, absorbance difference, processing absorption spectra. The aim of this review is to present information on simultaneous equation method, difference spectrophotometry, derivative spectrophotometry, absorbance ratio spectra, derivative ratio spectra, successive ratio - derivative spectra, Q-absorbance ratio method, absorptivity factor method, dual wavelength method, absorption factor method, multivariate chemometric methods and isobestic point methods. Ultraviolet-Visible spectroscopy is used to obtain the absorbance specra of compound in solution or as a solid.

KEYWORDS: *Ultraviolet spectroscopy, Simultaneous equation method, Derivative spectrophotometry, Derivative ratio spectra, Isosbestic point method, Multivariate chemometric methods*

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INTRODUCTION TO SPECTROSCOPY[12]

Spectroscopy as a science began with Isaac Newton splitting light with a prism and was called optics. Therefore, it was originally the study of visible light which we call color that later under the studies of James Clerk Maxwell came to include the entire electromagnetic spectrum. Spectroscopy is the branch of science dealing with the study of interaction of electromagnetic radiation with matter. The most important consequence of such interaction is

that energy is absorbed or emitted by the matter in discrete amounts called quanta. The absorption or emission processes are known throughout the electromagnetic spectrum ranging from the gamma region (nuclear resonance absorption or the Mossbauer effect) to the radio region (nuclear magnetic resonance). When the measurement of radiation frequency is done experimentally, it gives a value for the change of energy involved and from this one may draw the conclusion about the set of possible discrete energy levels of the matter. The ways in which the measurements of radiation frequency (emitted or absorbed) are made experimentally and the energy levels deduced from these comprise the practice of spectroscopy.

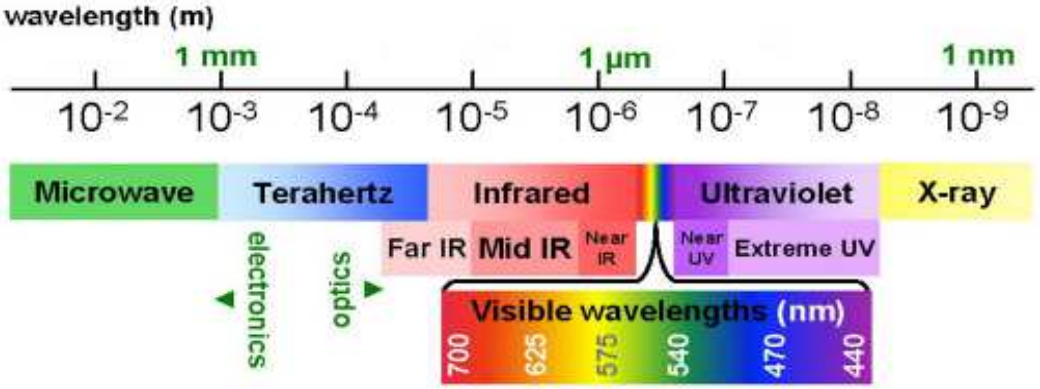


Figure 1: spectroscopy

1. Atomic spectroscopy

This deals with the interaction of electromagnetic radiations with atom which are most commonly in their lowest energy state called the ground state. The electronic absorption of electromagnetic radiation can occur only if the photon has an energy which is equal to the energy difference between two quantized energy levels, i.e., $\Delta E = h\nu$

where AE is the energy difference between two quantum levels and v is the frequency of photon which can result in the electronic excitation. Applications of electronic spectroscopy in the field of chemistry are few. However, its importance has increased by the development of lasers.

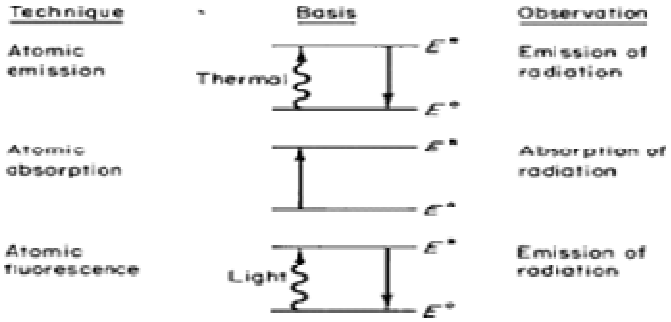


Figure 2: atomic spectroscopy

2. Molecular Spectroscopy

This deals with the interaction of electromagnetic radiation with molecules. This results in transitions between rotational and vibrational energy levels in addition to electronic transitions. As a result, the spectra of molecules are much more complicated than those of atoms. Molecular spectra extend from the visible through infrared into the microwave region. Current interest in molecular spectroscopy is very great because the number of known molecules are extremely large as compared with free atoms. The various types of spectra given by molecular species, the regions in which these spectra lie and the energy changes that takes place in the molecules on absorption of radiation, are listed below.

UV-Spectroscopy

Introduction

UV spectroscopy is the absorption or reflectance spectroscopy of the ultraviolet and adjacent visible regions of the electromagnetic spectrum. It is also known as UV-visible spectrophotometry (UV-Vis or UV/Vis). Because of its low cost and ease of implementation, this methodology is widely used in a wide range of applied and fundamental applications[3] It is a physical technique of the optical spectroscopy that uses light in visible ultraviolet, and near infrared ranges[1], the molecular absorbtion is studied in the region of 190 to 800nm [2]

PRINCIPLE

A molecule or ion will exhibit absorption in the visible or ultraviolet region when radiation causes an electronic transition within its structure. Thus, the absorption of light by a sample in the ultraviolet or visible region is accompanied by a change in the electronic state of the molecules in the sample. The energy supplied by the light will promote electrons from their [1]Basic principle of spectroscopy is the beer-lamberts law[4]

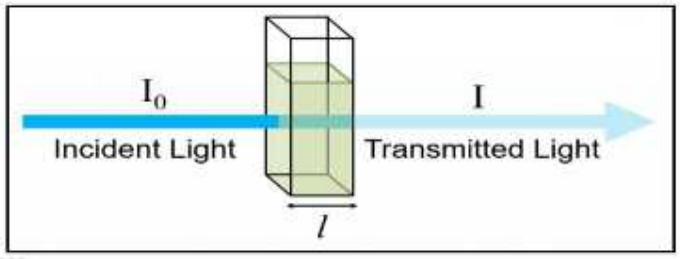


Figure 3: Mechanism of absorbance

Beer law

Beer's law state that absorbance is proportional to the concentration of the material sample[1].The intensity of the incident radiation (Io) will be higher than the emerging radiation when a beam of electromagnetic radiation passes through an absorbing material (I). The general rule known as Beer's law can be used to quantitatively describe how radiant energy is absorbed by materials. According to Beer's law, the amount of radiation that is absorbed (absorbance, A) or transmitted by a solution or medium is inversely related to the amount of the absorbing substance that is present, c (moles per liter), and the length of the radiation's passage through the

sample, b. (centimeters). Therefore, a plot of absorbance against concentration should result in a line that is straight and has a slope equal to Єb, passing through the origin[.13]

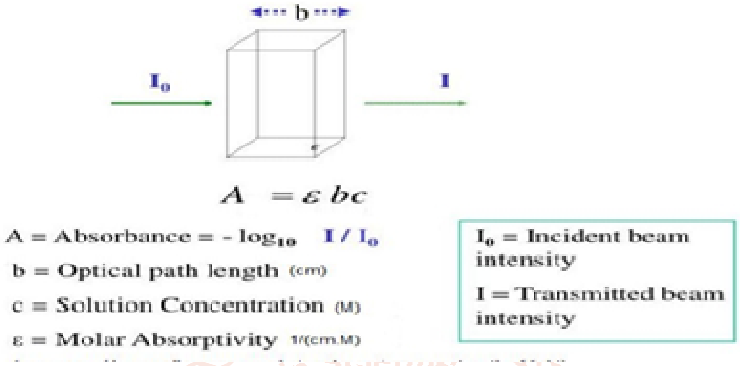
$$A = - \log (I/Io) = Єbc$$

where Є = k/2.303

The molar absorptivity is a constant ϵ that is unaffected by concentration or path length. If the route length and molar absorptivity are known, Beer's law equation can be used to calculate the concentration of an organic molecule by locating its highest absorbance in the UV-Vis absorption spectrum.

Lambert's law

Lambert's law that absorbance of a material is directly propotional to thickness (path lenth)[1]



ELECTRONIC TRANSITION[14]

Electroniv transirion in uv visible spectroscopy which are imporatant **n**→π***t**ransition & $\pi \rightarrow \pi$* **t**ransition in this an electron of unshared electron pair on hetero atom is excited to π* anti bonding orbital

1. $\sigma \rightarrow \sigma *$ transition
2. $\pi \rightarrow \pi$* transition
3. n→σ* transition
4. n→π*transition
5. $\sigma \rightarrow \pi *$transition
6. $\pi \rightarrow \sigma *$transition

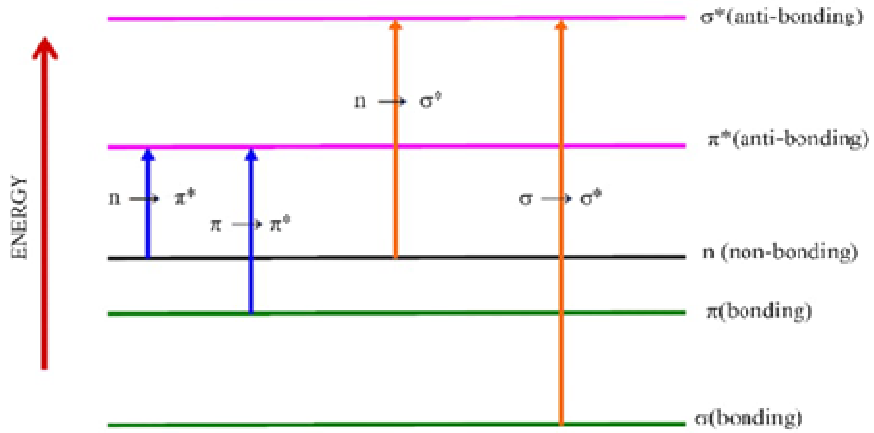


Fig 4: Graphical representation of Electronic transition

1. $\sigma \rightarrow \sigma *$ transition:

- σ electron from orbital is excited to corresponding antibonding $\sigma *$
- The energy required is large for this transition.
- Example : methane has C-H bond only and can undergo $\sigma \rightarrow \sigma *$ transition
- And shows absorbance maximum at 125nm.

2. $\pi \rightarrow \pi *$ transition:

- π electron in bonding orbital is excited to corresponding antibonding orbital π*

- compound containing multiple bonds like alkyens, carbonyl, nitriles, aromatic compounds etc. Undergo π→π* transition.

3. n→σ* transition

- Saturated compounds containing atoms with lone pair of electrons like O, N, S and halogens are capable of n → σ* transition.

4. n→π*transition

An electron from non-bonding orbital is promoted to anti-bonding π* orbital.

Compounds containing double bond involving hetero atoms (C=O, C=N, N=O) undergo such transitions.

5. σ→π*transtion and π→σ*transition

These electronic transitions are forbidden transitions & are only theoretically possible.

Thus, n→ л & л→л electronic transitions show absorption in region above 200 nm which is accessible to UV-visible spectrophotometer.

Concept of chromophore:

Many organic molecules absorb ultraviolet/visible radiation and this is usually because of the presence of a particular functional group. The groups that actually absorb the radiation are called chromophores.

Auxochrome

Auxochrome can be defined as any group which does not itself acts as a chromophore but whose presence brings about a shift of the absorption band towards the red end of the spectrum (longer wavelength).

Example: Auxochromic groups are –OH, -OR, NH_2, - NHR, -NR2, -SH,etc.

Spectral shift

Changes in chemical structure or the environment lead to changes in the absorption spectrum of molecules and materials. There are everal tones that are commonly used to describe these shifts,

Bathochromic effect,
Hypsochromic effect,
Hyperchromic effect,
Hypochromic effect

A. Bathochromic shift

The absorptions of two or more chromophores which are separated by more than one bond are usually additive, but when chromophores are conjugated, i.e. separated by a single bond, pronounced effects are produced. The maximum absorption is shifted to longer wavelengths, thus bringing it into the working range of spectrophotometers.

The effect, by virtue of which the absorption maximum is shifted towards longer wavelength due to the presence of an auxochrome or by the change of solvent, is called as Bathochromic shift or Red shift.

B. Hypsochromic shift

It is an effect by virtue of which the absorption maximum is shifted towards shorte wavelength. The absorption shifted towards shorter wavelength is called Blue shift or Hypsochromic shift

E.g. aniline has max 280nm

C. Hyperchromic shift:

It is an effect due to which the intensity of absorption maximum increases.

.Example

Pyridine Amas 257 пт, Селез 2750
methyl pyridine lambda max =262 nm. v max =3560
The introduction of an auxochrome usually increases intensity of absorption.

D. Hypochromic shift:

It is an effect due to which the intensity of absorption maximum decreases i.e. extinctioncoefficient, Emax decreases. The introduction of group which distorts the geometry of the molecule causes hypochromiceffect.

Example

Rightarrow lambda max =250 nmE max =19000

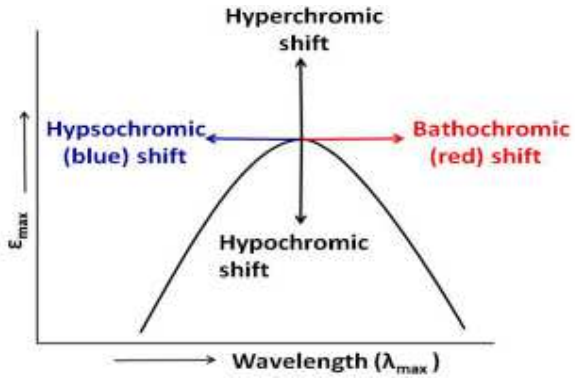


Fig5:spectral shifts in uv visible range

INSTRUMENTATION[6]

1. Source of light
2. Monochromator
3. Simple solution in cuvette
4. Detector
5. Readout device

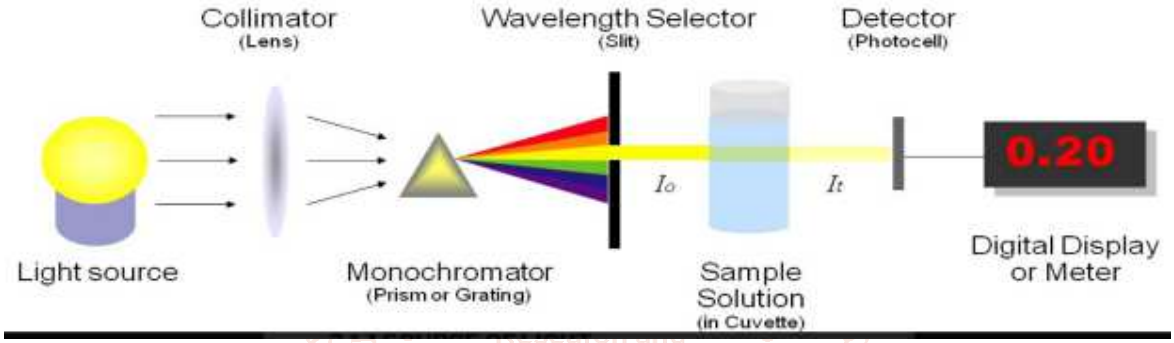


Fig6:Instrumentation of uv spectroscopy

1. Source of light:

The UV-Vis Spectroscopy requires a continuous source, or one that emits radiation over a broad range of wavelengths.

Various UV radiation sources are as follows:[7]

A. Hydrogen lamp:
B. Deuterium lamp
C. Tungsten lamp:
D. Xenon discharge lamp

A. Hydrogen lamp

Hydrogen lamp are stable, robust and emit continuous radiation in range of 160-380 nm.It Consist of hydrogen gas under high pressure through which there is electrical discharge, hydrogen molecules areexcited and emit radiation.

Fig 6 :Hydrogen lamp

B. Deuterium lamp:

A deuterium lamp is a gas discharge lamp and is often used as a UV source. It emits in radiation in range of 160-450nm.It is more expensive that Hydrogen lamp.

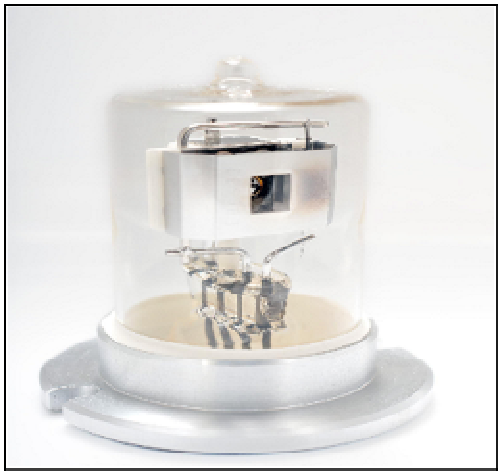


Fig 7:Deuterium lamp

C. Tungsten lamp:

Tungsten Lamp is the most common light source used in spectrophotometer. It consists of a tungsten filament enclosed in a glass envelope, with a wavelength range of about 330 to 900 nm, are used for the visible region.

Fig 8: Tungsten lamp:

D. Xenon discharge lamp:

A xenon lamp is a discharge light source with xenon gas sealed in a bulb. The xenon emits radiation in range of 250-600 nm.

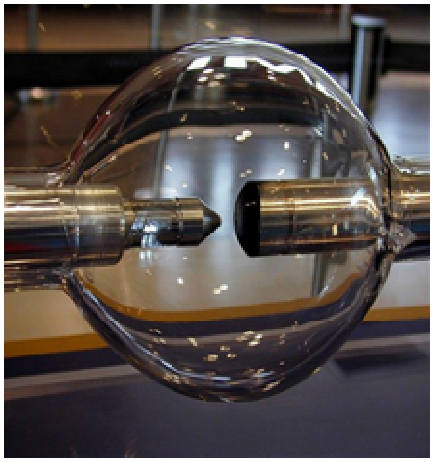


Fig 9:. Xenon discharge lamp:

2. Monochromator:[7,8]

A Monochromator is an optical device that transmits a mechanically selectable narrow band of wavelengths of light chosen from a wider range of wavelengths available at the input. And the unwanted radiations are blocked by the slit allowing only the desired ray to pass (monochromatic).

All Monochromator contain the following component parts:

- An entrance slit
- A collimating lens
- A dispersing device
- A focusing lens
- An exit slit

Radiation with many wavelengths, or polychromatic radiation, enters the monochromator through the entrance slit. After being collimated, the beam angles toward the dispersing component. The grating or prism separates the beam's wavelengths into their individual components. Only radiation of a specific wavelength exits the monochromator through the exit slit by changing the dispersing element or the exit slit.

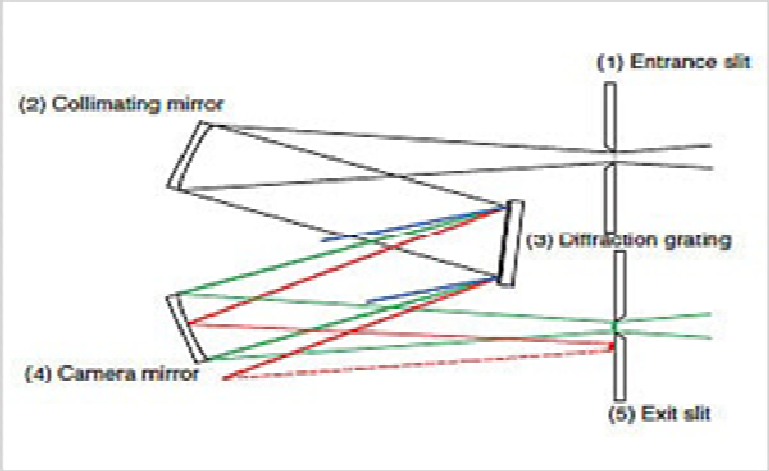


Fig 10: Proceses of monochromator

Types of monochromator:

1. Prism Monochromator

Based on refraction of light and fact that different wavelengths of radiation have different values of Refractive index in a medium.

2. Grating monochromator

Disperse ultraviolet, visible, and infrared radiation typically using replica gratings, which are manufactured from a master grating. A master grating consists of a hard, optically flat, surface that has a large number of parallel and closely spaced grooves.

3. Sample solution in cuvette [14,15]

Cuvette are sample container which used to hold samples for spectroscopic measurement and which is transparent to all wavelength of light passing through it. The cuvette made of Quartz, Square shape and having path length 1 cm are selected and can be used for wavelengths ranging from 190 to 200 nm

4. Detector:[14]

A detector Converts a light signal into an electrical signal.It should give a linear response a wide range of low noise and High sensitivity.

1. Photomultiplier tube detector.
2. Photodiode detector

A. Photomultiplier tube detector.

The photomultiplier tube is a commonly used detector in UV-Vis spectroscopy. It consists of a photoemissive cathode (a cathode which emits electrons when struck by photons of radiation), Anodes (which emit several electrons for each electron striking them).

A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the first Anode (which is 90V more positive than the cathode). The electrons strike the first anode, causing the emission of several electrons for each incident electron. These electrons are then accelerated towards the second anode, to produce more electrons which are accelerated towards the anode. The electrons are collected at the anode. By this time, each original photon has produced 106-107 electrons. The resulting current is amplified and measured. Photomultipliers are very sensitive to UV-visible radiation. They

have fast response times. Intense light damages photomultipliers; they are limited to measuring low power radiation.(1)

5. READOUT DEVICE.

Digital screen to record an uv spectrograph with absorbance against the Wavelength**.**

Different UV spectrophotometric multicomponent analysis methods[16]

1. Simultaneous equation method:

If a sample contains two absorbing drugs (x and y) each of which absorbs at the λ max of the other, it may be Possible to determine both drugs by the technique of simultaneous equation (Vierordt's method) provided that Certain criteria apply.The information required is The absorptivities of x at λ1 and λ2, ax1 and ax2 respectively The absorptivities of y at λ1 andλ2, ay1 and ay2 respectively The absorbance of the diluted samples at λ1 and λ2, A1 and A2 respectively. Let Cx and Cy be the concentration of x and y respectively in the diluted samples. Two equations are constructed Based upon the fact that at λ1, the absorbance of the mixture is the sum of the individual absorbance of x and y{5}

So, at λ_1 $A_1 = a_{x1}bc_x + a_{y1}bc_y$

At λ_2 $A_2 = ax_2bc_x + ay_2bc_y$

If cell is 1 cm, b = 1 equation 2 become,
$cy = (A-a_{x2}c_x)/a_{y2}$

Substituting value of cy in equation (1), thus
$a_{x1}bc_x = A-a_{y1}c_y$

$c_x = (A_2\ a_{y1}-A_1\ a_{y2})/(a_{x2}a_{y1}-a_{x1}a_{y2})$

Similarly for c_y

$cy = (A_1\ a_{x2}-A_2\ a_{x1})/(a_{x2}a_{y1}-a_{x1}a_{y2})$

Glenn" have been suggested criteria for obtaining maximum precision, based on absorbance ratio that place limit on the relative concentration of the component of the mixture. The criteria for that ratio should lie outside the range 0.1–2 for precise determination of X and Y, respectively.

Condition to fulfill this criteria:

- λmax of two-component should be reasonably dissimilar.
- Two-component should not interact chemically, thereby negating the initial assumption that the absorbance.

2. Difference spectrophotometry:

The selectivity and accuracy of spectrophotometric analysis of samples containing absorbing interferents may be markedly improved by the technique of difference spectrophotometry. The essential feature of this method is that the measured value is the absorbance difference (A) between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics.

3. Derivative spectrophotometry (DS):

DS involves the conversion of a normal spectrum (fundamental, zero order spectrum) to its first, second or higher derivative spectra by differentiating absorbance of the sample with respect to wavelength□. The differentiation of zero-order spectrum can lead to separation of overlapped signals, elimination of background caused by presence of other compounds in a sample, improvement of resolution of mixtures as it enhances the detectability of minor spectral features, and enhancement of sensitivity and specificity.

4. Absorbace ratio spectra method:

Consider a mixture of two compounds x and y. The absorption spectrum of the mixture "measured in 1 cm cell" is defined by the equation

$$A_M = a_x C_x + a_y C_y$$

Where; AM is the absorbance of the mixture, ax and ay are the molar absorptivities, Cx and Cy are the concentrations of x and y, respectively. If the absorbance of the mixture is divided by the absorbance of a standard solution of x (its absorbance A0 X = ax C 0 X), the following equation results

$$\frac{A_M}{A_X^0} = \frac{C_X}{C_X^0} + \frac{A_y}{A_X^0}..$$

The ratio is $\frac{C_X}{C_X^0}$ a constant value which can be eliminated by taking the difference in absorbance ratio amplitudes between two wavelengths λ 1 and λ 2

5. Derivative ratio spectra method:

This simple spectrophotometric method, developed by Salinas et al. Is based on the derivation of the ratio spectra for resolving binary mixtures. It permits the use of the wavelength of highest value of analytical signals with several maxima and minima, which give an opportunity for the determination of active compounds in the presence of other compounds and excipients which could possibly interfere in the assay

6. Double divisor ratio spectra derivative method:

This method is based on the use of the derivative of the ratio spectrum obtained by dividing the absorption spectrum of the ternary mixture by a standard spectrum of a mixture of two of the three compounds in the mixture, and the measuring at either the maximum or minimum wavelengths.

7. Successive ratio - derivative spectra method:

This method is used for simultaneous determination of the three compounds in ternary mixtures without need to know the ratio of concentration of species.

8. Q-absorbance ratio method:

This method also termed "**absorption ratio method**" is a modification of the simultaneous equation's method. According to this method, the ratio of absorbance at any two wavelengths for a substance, which obeys Beer's law, is a constant value independent of the concentration and path length. This constant is termed as "Hufner's Quotient" or Q-value. The method involves the measurement of absorbance at two wavelengths, one being the λ max of one of the components (λ 2) and the other being a wavelength of equal absorptivity of the two components (λ 1), called the iso-absorptive point. The concentration of each component can be calculated by mathematical equations

Cx = (Qm-Qy/Qx-Qy) * A/a1 …… (1)

Cy = (Qm-Qx/Qy-Qx) * A/a2 …… (2)

where; Cx and Cy are the concentrations of x and y respectively, A is absorbance of sample at isoabsorpitive wavelength and a1 and a2 are the absorptivity of x and y respectively at isoabsorpitive wavelength.

9. Isosbestic "isoabsorptive" point method:

Erram and Tipnis developed the isosbestic point method. This technique can be used only if the spectra of the same concentration of the two studied drugs cross at a point called isosbestic or isoabsorptivity point. At the isosbestic point both drugs have equal absorptivities and their mixture acts as a single component and gives the same absorbance as pure drug.

10. Absorpitivity factor method:

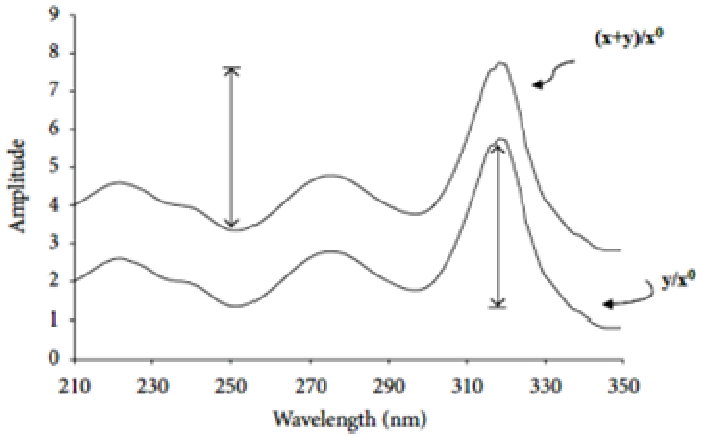


Figure 8 :- Zero order spectra of 8 µg. mL-1 salmeterol and 16 µg. mL-1 fluticasone showing the absorptivity factor points.

The absorptivity factor (modification of the classical isoabsorpitive method) is applied for the analysis of binary mixture if only there is a large difference in the absorptivity between both drugs, so there is no occurrence of an isoabsorptive point.

In isoabsorptive technique the spectra of the same concentration of the two studied drugs should cross at a point called isoabsorptivity point at which they have equal absorptivities while in absorpitivity factor method the crossing point did not occur at equal concentration. Crossing point is obtained only between different concentrations of the two drugs at which the absorptivities of the two drugs are not equal but they are equal to the inverse of the ratio of the used concentrations.

11. Dual wavelength method:

Dual wavelength method "also known as two wavelengths method" facilitates analyzing a component in presence of an interfering component by measuring the absorbance difference between two points in the mixture spectrum. In this method (Fig. 4); one of the drugs is considered as a component of interest and the other drug is considered as an interfering component and vice-versa. The basis for such method is the selection of two wavelengths where the interfering component shows the same absorbance □A equals zero) whereas the component of interest shows significant difference in absorbance with concentration. □A between two points on the mixture spectra is directly proportional to the concentration of the component of interest independent of interfering component. This method was used for simultaneous determination of different drugs, e.g., atenolol and indapamide, drotaverine and aceclofenac, atorvastatin and ezetimibe, chlorpheniramine and phenylpropanolamine, dexketoprofen and tramadol

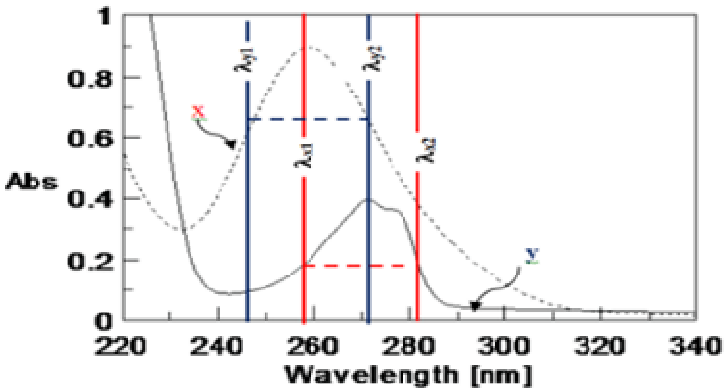


figure 9 :- Selection of wavelengths for dual wavelength method

12. Mean centering of the ratio spectra:

This method is applied for further improvement of the selectivity to resolve the overlap present between drugs in binary and ternary mixtures. This eliminates the derivative step and therefore the signal-to-noise ratio is enhanced.

13. Absorption factor method (AFM):

This method describes the analysis of a binary mixture where the two components x and y have overlapped spectra. Y shows interference at λ max of x, while x shows no interference with y at another wavelength (λ 2).

14. Multivariate chemometric methods:

Chemometrics, in the most general sense, is the art of processing data with various numerical techniques in order to extract useful information. Drug separation, identification, determination and validation have been studied using chemometrics. Chemometrics recognizes that it is often better to measure many nonselective signals and then combine them in multivariate model (multivariate analysis), whereby multiple variables are considered simultaneously. A multivariate measurement is defined as one in which multiple measurements are made on a sample of interest. So, more than one variable or response are measured for each sample. Multivariate methods include multiple linear regression (MLR) methods and factor-based methods.

ADVANTAGES[5]

- The core advantage is the accuracy of the UV-VIS spectrophotometer
- Easy to handle
- Cost effective
- The UV-VIS spectrometer is easy to handling and use
- Provide robust operation
- UV-VIS spectroscopy is simple to operate
- Cost effective instrument
- Cover the entire of ultraviolet and visible
- It can be utilized in the qualitative and quantitative analysis
- The Derivative graph can be obtained by UV-VIS spectrophotometer

- It can be used in the degradation study of drug Only possible for the analytes which have a chromophore
- Simple design easy process control high gain.
- Low dark current quik time response high sensitivity & quantum efficiency.
- Fast time response, high impedance, low dark current, low bias operation, high-frequency operation, easier fabrication
- Fast time response, minimally affected by bias, simple fabrication process, low cost, easy integration
- UV spectrophotometer is a highly simple instrument which makes it easier to couple with other analytical instrument such as RP-HPLC

DISADVANTAGES:[17]

- Only those molecules are analyzed which have chromophores
- The results of the absorption can be affected by pH, temperature,
- contaminants, and impurities.
- Only liquid samples are possible to analyze
- It takes time to get ready to use it
- Cuvette handling can affect the reading of the sample
- Lack of sensitivity & selectivity
- Limited to uv visible absorbing compound
- Mixture of molecules can be a problem due to overlap
- Spectra are not highly specific for particular molecule
- Absorption can be dependent on solution conditions

APPLICATIONS[18]

UV –vis spectroscopy has many different application

1. Detection of impurities
2. Structural elucidation of organic compounds
3. Quantitative analysis
4. Qualitative analysis
5. Chemical analysis
6. Quantitative analysis of pharmaceutical substance
7. Dissociation constant of acids and bases
8. Molecular weight determination
9. As HPLC detector
10. Deviations from the Beer-Lambert law

Conclusion:

UV-Vis Spectroscopy is an important technique for studying the optical proprties of Pharmaceutical drugs. Uv Visible Spectroscopy is based on firm, more selective, efficient, fast & reprodusible anlytical method can be developed. The pharmaceutical analysis by UV-Spectroscopy comprises the procedure necessary to determine the “Identity, strenth, quality & purity” of compounds. In general terms, there are two major measurement techniques; how much analyte is in the sample (quantitative analysis) and which analyte is in the sample (qualitative analysis).

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